AWARD NUMBER: W81XWH-15-2-0033

TITLE: Identifying New Chemical Entities that Treat and Prevent Relapsing Vivax and Drug-Resistant Falciparum Malaria in U.S. Military Personnel

PRINCIPAL INVESTIGATOR: David A. Fidock

CONTRACTING ORGANIZATION: Trustees of Columbia University
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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland, 21702-5012

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14. ABSTRACT

Our project has now nearly completing the screening of 400,000 compounds from the National Center for Advancing Translational Sciences (NCATS) chemical library, for activity against *Plasmodium falciparum* asexual blood stages. To date we have nearly 2,000 compounds that inhibit growth at low to submicromolar concentrations Nearly 600 of our prioritized hit compounds have been tested against rodent malaria liver stages and we have a set of 43 active compounds that have promising potency and selectivity from which to initiate *in vivo* assays to assess for cure and prophylaxis. We have also made progress with generating plasmids to engineer *Plasmodium cynomolgi* parasites that can be used to screen for activity against liver stages in a model of relapsing malaria. We are on track with our goal to identify chemical series that can be tested as candidate medicines to cure and prevent malaria in US Military personnel.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

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1. Introduction:

The goal of this project is to identify novel chemical compounds that are active against the blood and liver stage forms of malaria parasites and that are useful for both prophylaxis and treatment of *Plasmodium vivax* and *Plasmodium falciparum* infections. Malaria has been identified as one of the most significant threats to deployed troops worldwide as this disease is endemic to Southwest Asia including Afghanistan, Southeast Asia, Africa, the Middle East, the Pacific, and both Central and South America. The project combines expertise from the Walter Reed Army Institute of Research (WRAIR) as the Partnering Institution, Columbia University as the Initiating Institution, and the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH) as a subsite affiliated with the WRAIR award. These teams combine chemistry, pharmacology and molecular and cellular parasitology to pursue a "hits to lead" program, whose primary objective is to generate and characterize compounds that could be developed into new medicines to treat and prevent malaria in US Military personnel.

2. Keywords:

Malaria, *Plasmodium falciparum*, *P. cynomolgi*, asexual blood stages, liver stages, high-throughput screen, drug assays, cell culture, transfection, green fluorescent protein.

3. Accomplishments:

3.1. Major Goals:

Our accepted statement of work (SOW) listed the following specific aims and tasks as part of our Year 1 work:

Specific Aim 1: Perform a high throughput screen (HTS)-based identification of antimalarial compounds. Our Major Task 1.1 was to confirm initial 2045 hits from first screen of 250,000 compounds. We proposed to conduct this work in the first three months, and this was successful. Our Milestone #1 was to move the first set of hits into downstream screens by the 4th month. That was also successful as we began to assay our hits against mammalian HepG2 cells to begin to test for selectivity against malaria parasites. Our Major Task 1.2 was to implement an HTS with an additional 150,000 compounds and confirm hits. We proposed to achieve this by the end of year 1 and this is 95% complete. All compounds have been screened, and we are now completing the last set of confirmatory screens with a set of 4,300 compounds. Our Milestone #2 was to move our second set of confirmed hits into downstream screens, which we estimated that we could meet by the end of year 1. Here also, we have met our milestone as these compounds have been tested for activity against HepG2 cells.

Specific Aim 2: Screen for inhibitors of rodent liver stage parasites *in vitro*. Our Major Task 2.1 was to identify compounds that selectively inhibit *P. berghei* liver stage parasites *in vitro* at submicromolar concentrations. Our Subtask 1 was to perform *in vitro* liver stage screens. Our Subtask 2 was to screen out non-selective compounds that inhibit HepG2 cells. Our timeline for this Aim was the first 15 months. We are on track with this, having already screened about 560 blood stage-active compounds against *P. berghei* liver stages. Our Milestone #3 was to define a list of compounds with parasite-specific sub-micromolar *in vitro* liver stage activity, which we estimated to achieve by month 15. We already have 43 compounds that are liver-stage active and expect to have more in the coming months.

As part of our statement of work (SOW), we also proposed to have local IACUC approval at CUMC and WRAIR by the end of month 3 and to have ACURO approval by the end of month 6. IACUC approval of our animal protocol (AN-AAA5200) was given by CUMC on 08/22/2015, prior to the beginning of the award. IACUC approval had already been obtained by WRAIR. An ACURO document was submitted 10/29/2015 and approved on 12/28/2015, signed by Colonel Bryan Ketzenberger, Director of the Animal Care and Use Review Officer at the US Army.

Specific Aim 3: Test hits for *in vivo* prophylaxis and blood stage cure in rodents. Our Major Task 3.1 was to triage hits, assess toxicity and metabolism. Our Subtask 1 was to triage out known metabolic liabilities and toxicophores. Our Subtask 2 was for the remaining pharmacophores, to assess toxicity and metabolism. We expected to complete this work during months 4–18 and a result we are half way through. Our work on this, described below, is about 50% complete. Our Milestone #5 was to finalize a list of hits with acceptable pharmacophores, with a stated goal of achieving this by the 18th month. This work is ongoing. Our Major Task 3.2 was to test hits for *in vivo* activity against *P. berghei* blood stages in mice. Our Major Task 3.3 was to test hits for *in vivo* activity against *P. berghe*i liver stages in mice. Both of these tasks were expected to begin in the 7th month and extend to month 21. Our Milestone #6 was to define hits with in evidence of *vivo* curative and prophylactic activity. We have not yet started major tasks 3.2 and 3.3 as we plan to first complete our HTS work and initial screens against HepG2 cells and liver stage parasites. We are nonetheless on track to achieve these goals by the 21st month as planned.

Specific Aim 4: Test down-selected hits for *in vitro* activity against *P. cynomolgi* proliferating and hypnozoite liver stages. Our Major Task 4.1 was to develop *P. cynomolgi* constructs and reporter lines for compound screens. Our Subtask 1 was to generate plasmids (months 7–10). Our Subtask 2 was to begin *in vivo* selection studies to obtain a reporter line (months 11–15). Our Milestone #8 was to obtain a GFP-luciferase *P. cynomolgi* reporter line by the 15th month. We have accomplished our subtask 1 and plan to begin subtask 2 in the next 3 months. The remainder of Specific Aim 4 and Specific Aim 5 are slated to begin in year 2 and have not yet begun.

3.2. Accomplishments made under these goals:

3.2.1. Major activities: Our major focus this first year has been to complete the HTS work of the NCATS collection of 400,000 compounds. This has been a large body of work that is nearly complete. First, we examined our set of ~250,000 compounds that had earlier been screened against the Dd2 strain of P. falciparum asexual blood stage parasites. Those initial data were generated using a luciferase-based method and yielded 2,045 hits that showed activity. We then retested 1,882 compounds at NCATS. The remaining 163 were not tested because either they represented compounds of known activity, or had undesirable chemical functionality, or could not be sourced. These 1,882 compounds were tested in a first run of 168 compounds in order to re-establish the conditions for our HTS. The second run tested 1,714 compounds. Parasites were procured through an internal collaborative arrangement between scientists at NCATS and the NIH. Overall, 659 showed an IC50 value < 2 μM. We also initiated a screen of the additional 150,000 compounds present in the current NCATS collection. Compounds were first tested in a five-point dilution series (with 5–fold dilutions), and active compounds retested in an 11-point 2–fold dilution series. From this, we obtained a set of 4,300 compounds with IC_{50} values < 2 μM that are now being retested in one final set of confirmatory assays. We expect those studies to be completed by the end of 2016.

Active compounds have also been screened against *P. berghei* liver stages cultured *in vitro*, using a parasite line expressing green fluorescent protein (GFP). GFP signals were examined at 44 hr post-inoculation, corresponding to mature liver stage parasites. From our first set of 250,000 compounds that yielded 659 active compounds, we selected 560 that were tested against *P. berghei* liver stages at single concentrations of 1 and 3 μM. In parallel, these were tested against HepG2 cells to assay for toxicity against mammalian cells. Based on these results and chemoinformatic analysis, we chose a subset of 44 potent and selective compounds for IC_{50} determination. Results showed that 43 of these compounds were active against liver stages with IC_{50} values below 1 μM (**Table 1**). IC_{50} values were

as low as 0.4 nM, indicating exceptional potency. We expect to have close to another 600 compounds from our final set of confirmatory screens that we can move into *P. berghei in vitro* liver stage screens.

IC_{50} values (μM)		
Pb Liver	**Pf ABS**	**HepG2**
0.0004	2.00	50.0
0.0006	1.58	50.0
0.0006	0.28	28.2
0.0060	1.41	50.0
0.0062	0.45	50.0
0.0063	0.89	50.0
0.0093	0.89	50.0
0.0099	1.58	50.0
0.012	0.63	50.0
0.013	1.58	22.4
0.013	2.00	50.0
0.015	2.00	50.0
0.016	0.71	35.5
0.023	1.58	50.0
0.023	0.79	50.0
0.024	0.56	35.5
0.024	2.00	50.0
0.027	0.50	50.0
0.029	1.26	22.4
0.039	1.00	50.0
0.048	2.00	50.0
0.065	1.78	50.0
0.099	0.25	50.0
0.11	0.50	50.0
0.12	0.63	14.1
0.14	1.78	50.0
0.14	1.58	50.0
0.15	1.58	11.2
0.16	0.89	50.0
0.18	0.13	28.2
0.19	0.79	15.8
0.19	0.79	31.6
0.23	0.22	39.8
0.25	0.89	50.0
0.26	0.71	11.2
0.27	0.45	10.0
0.40	0.56	28.2
0.41	0.35	50.0
0.49	1.58	50.0
0.61	1.26	39.8
0.66	0.50	50.0
0.67	0.45	12.6
0.94	0.63	15.8

Table 1. IC_{50} values of our first set of 43 compounds with submicromolar activity against *P. berghei* liver stage parasites. Most of these are also potent against P. *falciparum* ABS parasites (Dd2 strain). All compounds have IC_{50} values > 10 μM against mammalian HepG2 cells, implying specific anti-parasitic activity. 10 and 25 compounds have liver stage IC_{50} values < 10 nM and 100 nM respectively, showing that we have outstanding potency among our compounds. These compounds are being tested for microsomal stability, permeability and solubility as part of our chemoinformatic profiling and triaging. Representatives will enter downstream testing, with more compounds becoming available soon.

We have also made good progress in constructing DNA plasmids that can be used to generate recombinant *P. cynomolgi* parasites that express GFP and luciferase. That line can then be used for luciferase or GFP-based *in vitro* screening of compound activity against *P. cynomolgi* liver stage parasites. To generate this plasmid, we customized a pair of zinc-finger nucleases (ZFNs) that specifically recognize the fatty acid elongase-1 (elo1) gene that we know in P. falciparum and P. berghei is non-essential and that we predict will similarly be non-essential in P. cynomolgi. We then created a DNA plasmid that expresses these two ZFNs, under the control of a single *calmodulin* gene promoter (**Figure 1A**). This plasmid also contains a GFP-luciferase (GFP-LUC) fusion that is linked to the human dihydrofolate reductase (h*dhfr*) selectable marker that mediates resistance to the antimalarial drug pyrimethamine. Our current plasmid uses the *hsp70* promoter that is expressed in liver-stage and ABS parasites, making it possible to select for parasites transformed with this plasmid in blood stages and to use it as a phenotypic readout in liver stage assays. We will use this plasmid to introduce our GFP-LUC expression cassette into the *P. cynomolgi* elo1 gene (**Figure 1B**).

3.2.2. Specific objectives: We have met our specific objective to screen a library of 400,000 compounds and identify hits that are active against P. falciparum ABS parasites. This work is nearly complete, as detailed above. We have also achieved our objective of generating a DNA plasmid for later work to screen against *P. cynomolgi* liver stage parasites, which contain both actively replicating forms as well as hypnozoites.

3.2.3. Significant results and key outcomes: These are described above.

3.2.4. Other achievements: Nothing to report.

3.3. Training and professional development opportunities: Nothing to report.

3.4. Dissemination of results to communities of interest: Nothing to report.

3.5. Plans during next reporting period to accomplish goals: Our next goal is to complete the confirmation screening of the last set of hits from the 400,000 compound library, which we expect will yield another 600 or so compounds with IC_{50} values against *P. falciparum* ABS parasites below 2 μM. We will also complete our HepG2 assays against these and proceed to testing against *P. berghei* liver stage parasites. From that list we hope to obtain another 50 or so compounds that show submicromolar activity against liver stage parasites. We plan to test all our active compounds (that should total close to 100) for their chemical and pharmacological properties, notably toxicity against HepG2 cells, solubility, permeability, and metabolic stability. Based on these results, we will select the most promising compounds and examine whether analogs are available that can be further tested for activity against ABS and liver stage parasites. From this set, we will then proceed with *in vivo* experiments in mice, using the modified Thompson model used by WRAIR to assess compound efficacy against blood stage *P. berghei* parasites. We will also test for activity against liver stage parasites using the *P. berghei* GFP-LUC strain that enables us to perform *in vivo* imaging of the liver stage burden in drug-treated mice, using the In Vivo Imaging System (IVIS) from Perkin Elmer. Those data will inform us which compounds can progress into in vitro testing against *P. cynomolgi* liver stage parasites.

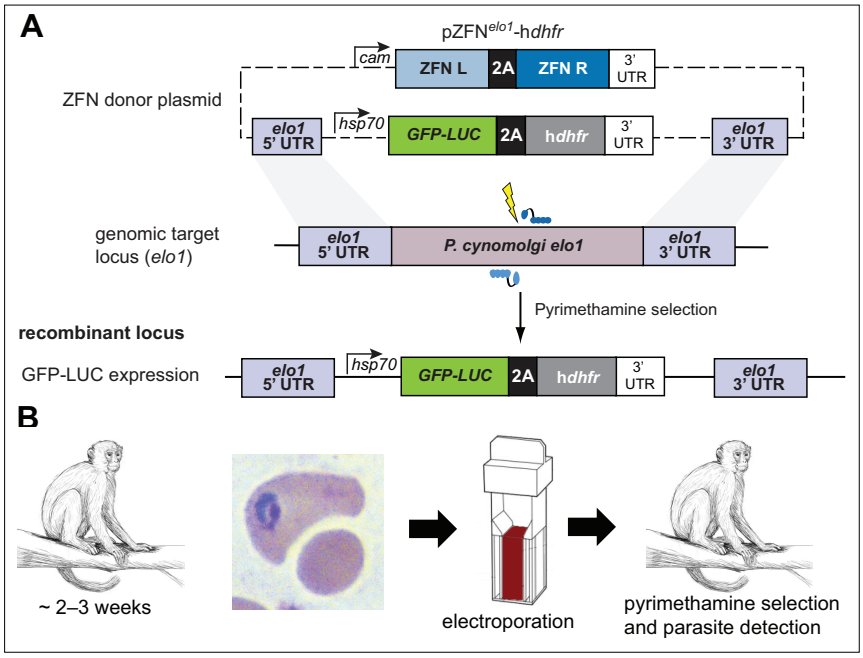


Figure 1. Method of generating a *P. cynomolgi* reporter line expressing GFP-LUC. (A) Plasmid used to generate a *P. cynomolgi* reporter line expressing GFP-LUC. This reporter is expressed by the *hsp70* promoter that is active in ABS and liver stages, including hypnozoites.. This reporter will be introduced into the *elo1* locus following a DNA double stranded break caused by *elo1*-specific ZFNs. **(B)** Transfection and selection conditions will follow procedures that have been successful with *P. cynomolgi* and *P. vivax* in monkeys.

In parallel, we plan to initiate transfection studies with *P. cynomolgi* parasites in rhesus macaques, using the plasmid described in **Figure 1**. If that experiment is successful then we will have a line that can be used for GFP or luciferase-based analysis of *in vitro* drug assays with our *P. cynomolgi* parasites. If we do not obtain these parasites then we will modify our plasmid strategy, for example by changing the promoter used to express the GFP-luciferase expression cassette or targeting a gene other than *elo1*.

4. Impact:

4.1. Impact on development of principal discipline of the project: Nothing to report.

4.2. Impact on other disciplines: Nothing to report.

4.3. Impact on technology transfer: Nothing to report.

4.4. Impact on society beyond science and technology: Nothing to report.

5. Changes/Problems:
5.1. Changes in approach and reasons for change: Nothing to report.

5.2. Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report. We are on track with our statement of work, timeline and milestones.

5.3. Changes that had a significant impact on expenditures: NCATS assumed some of the pharmacological testing (including tests with HepG2 cells and assays for compound solubility, permeability and metabolic stability) that was originally planned for WRAIR. NCATS personnel are separately funded and the NCATS budget of $100,000 was for supplies. In alignment with this change, WRAIR has not yet hired a research laboratory technician as of yet, leading to a positive balance. Additional work by WRAIR in year 2 is expected to spend out this balance.

5.4. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

6. Products:
6.1. Publications, conference papers and presentations: Nothing to report.

6.2. Websites: Nothing to report.

6.3. Technologies or techniques: This project has enabled NCATS to optimize their quantitative HTS studies with cultured *P. falciparum* ABS parasites that allows them to derive IC_{50} values for hundreds of thousands of compounds in a period of several months. These data provide this project with an outstanding set of novel compounds to drive our malaria drug discovery program.

6.4. Inventions, patent applications and/or licenses: Nothing to report.

6.5. Other products: Our project shares and regularly updates a file that lists compound structures and names, blood and liver stage activity, IC_{50} values for parasites and HepG2 cells, and pharmacological properties. We are constructing DNA plasmids that will be used to generate recombinant *P. cynomolgi* parasites for assessment of compound activity against proliferating or hypnozoite liver stage parasites.

7. Participants & Other collaborating organizations:
7.1. Individuals that have worked on the project:

Name	David Fidock (CUMC)
Project role	Initiating PI
Research identifier (ORCID)	0000-0001-6753-8938
Nearest person month worked	1
Contribution to project	Led project, managed studies by Fidock lab, organized monthly teleconference calls and distributed minutes, prepared quarterly and annual reports.
Funding support	PRMRP, NIH, Bill & Melinda Gates Foundation

Name	Philipp Henrich (CUMC)
Project role	Postdoctoral Scientist
Research identifier (ORCID)	None
Nearest person month worked	10
Contribution to project	Worked on DNA plasmids and *in vitro* parasite studies with compounds.
Funding support	CDMRP, NIH

Name	Santha K. Tiruppadiripuliyur (CUMC)
Project role	Postdoctoral Scientist
Research identifier (ORCID)	None
Nearest person month worked	7
Contribution to project	Worked on *in vitro* parasite studies with compounds.
Funding support	CDMRP, NIH

Name	LTC Norman Waters (WRAIR)
Project role	Partnering PI
Research identifier (ORCID)	0000-0002-0724-5823
Nearest person month worked	1
Contribution to project	Managed WRAIR contribution to project on pharmacology and compound testing.
Funding support	US Department of the Army

Name	LTC Mark Hickman (WRAIR)
Project role	Co-Partnering PI
Research identifier (ORCID)	0000-0001-8183-2076
Nearest person month worked	1
Contribution to project	Performed chemoinformatic analysis of active compounds.
Funding support	US Department of the Army

Name	Dr. Richard Sciotti (WRAIR)
Project role	Medicinal Chemist
Research identifier (ORCID)	None
Nearest person month worked	2
Contribution to project	Medicinal chemistry of promising hits.
Funding support	US Department of the Army

Name	Ajit Jadhav (NCATS)
Project role	Project manager at NCATS subsite
Research identifier (ORCID)	Not available
Nearest person month worked	1

Contribution to project	Managed project resources including personnel and lab operations.
Funding support	NIH/NCATS

Name	Bryan Mott (NCATS)
Project role	Staff chemist
Research identifier (ORCID)	None
Nearest person month worked	5
Contribution to project	Planned high-throughput screens (HTS), analysed results, managed compound procurement and distribution, tabulated data.
Funding support	NIH/NCATS

Name	George Djorbal
Project role	Staff Biologist
Research identifier (ORCID)	None
Nearest person month worked	2
Contribution to project	Conducted HTS.
Funding support	NIH/NCATS

Name	Richard T. Eastman
Project role	Postdoctoral Scientist
Research identifier (ORCID)	None
Nearest person month worked	2
Contribution to project	Conducted HTS.
Funding support	NIH/NCATS

7.2. Change in active other support of the PD/PI or senior/key personnel since the last reporting period:

A change in other support applies to the key personnel listed below:

Dr. David Fidock, Initiating PI:
Dr. Fidock has two new grants since the current PR140137 application was submitted. These grants do not reduce the level of effort Dr. Fidock is dedicating to the current PR140137 project.

Title:	Elucidating the molecular basis of piperaquine resistance and the role of altered hemoglobin metabolism in *Plasmodium falciparum* (R01 AI124678)
Agency:	NIAID/NIH
Officer:	Dr. Glen McGugan. E-mail: gmcgugan@mail.nih.gov
Period:	02/01/16 – 01/31/21
Funding:	$254,156 direct costs per year (Fidock lab)

Goals: This project aims to define the mechanistic basis of piperaquine resistance in *Plasmodium falciparum* parasites, using a combination of forward and reverse genetic techniques.

Aims: 1) Test the hypothesis that genes regulating hemoglobin metabolism and digestive vacuole transport define piperaquine resistance in Cambodian isolates; 2) Implement genetic crosses to map piperaquine resistance in isolates from French Guiana and use transfection to confirm the causal genes; and 3) Define the functional basis of piperaquine resistance and its impact on other antimalarials.

Role: Principal Investigator

Overlap: None

Title: Function of Antimalarial Drug Resistance Proteins
Commitment: 1.2 calendar months per year
Agency: NIAID/NIH
Officer: Dr. Michael O'Neil. E-mail: michael.o'neil@nih.gov
Period: 12/01/15 – 11/30/20
Funding: $75,153 direct costs per year (Fidock lab)
Goals: Define the contribution of mutant PfCRT and PfMDR1 isoforms to multidrug resistance in *P. falciparum* asexual blood-stage parasites
Aims: 1) Elucidation of PfCRT functional diversity; 2) Elucidation of PfMDR1 functional diversity; and 3) Elucidating the structure of PfCRT and PfMDR1.
Role: Subaward Investigator (Principal Investigator Dr. P. Roepe, Georgetown University)

The NIH R01AI05234 grant held by Dr. Fidock as PI is now in a period of no-cost extension since 07/01/2016 and Dr. Fidock's effort on this grant has decreased from 2.4 to 1.6 calendar months per year.

LTC Norman Waters, Partnering PI:
The project listed below has now been extended from its earlier end date of 2014 and remains active.

Title: Surveillance of malaria drug resistance in the South Pacific
Commitment: 50%
Agency: Department of Defence, Global Emerging Infections Surveillance and Response System
Period: 2008 – 2016
Funding: $1113,000
Goal: Determine level of antimalarial drug sensitivity in *P. falciparum* and *P. vivax* within the Solomon Islands and Vanuatu.
Aims: 1) Determine the prevalence of malaria within remote regions of the South Pacific; 2) Collect approximately 10,000 samples for genotypic studies; 3) Determine the prevalence of drug resistant polymorphism in several validated molecular markers; 4) Identify the clonality and origin of malaria parasite to determine drug resistant foci and genetic drift; and 5) Develop a model that defines how malaria drug resistance spreads throughout the South Pacific Region.
Role: Principal Investigator

The project listed below has ended since the time of the approved PRMRP submission.

Title:	The control and regulatory mechanisms of artemisinin induced dormancy in *P. falciparum*
Commitment:	20%
Agency:	National Health and Medical Research Council
Period:	2012 – 2015
Funding:	$457,000
Goal:	To understand the cell cycle regulatory mechanisms involved in the induction, maintenance and recovery of artemisinin induced dormancy.
Aims:	1) Investigate the expression and activity of CDKs and cyclins in normal or dormant parasites; 2) Investigate the role of a G_1 cell cycle checkpoint in the induction of ART-induced dormancy; and 3) Investigate the role of CDKs in the induction and recovery of ART- induced dormancy.
Role:	Co-Principal Investigator

7.3. Other organizations involved as partners: Nothing to report.

8: Special reporting requirements:
8.1. Collaborative Awards: the Initiating PI Dr. David Fidock and the Partnering PI LTC Norman Waters are providing independent annual reports for this project (W81XWH-15-2-003 and W81XWH-15-2-0034 respectively). Each report has its own separate cover page, SF298 and quad chart.

8.2. Quad Chart: Please see next page. The quad chart for LTC Waters is provided in that separate report.

9: Appendices: None.

Identifying New Chemical Entities that Treat and Prevent Relapsing vivax and Drug-Resistant falciparum Malaria in U.S. Military Personnel

PR140137

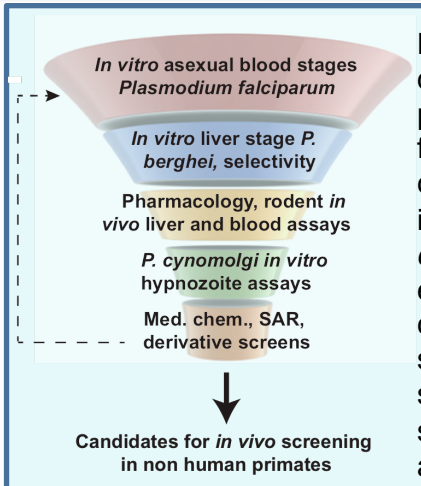
PI: David A. Fidock **Organization:** Columbia University **Award Amount:** $880,000

Study/Product Aim(s)

- Aim 1: Identify antimalarials using a high-throughput screen (HTS) against *Plasmodium falciparum* asexual blood stages.
- Aim 2: Screen for selective inhibitors of rodent liver stage parasites *in vitro.*
- Aim 3: Test hits for suitable pharmacological properties and *in vivo* efficacy as prophylactic and curative agents in rodent malaria models.
- Aim 4: Identify inhibitors of *P. cynomolgi* liver stages in *vitro*.
- Aim 5: Optimize hits, evaluate derivatives *in vivo* and *in vitro.*

Approach

Our project, involving Columbia University, the Walter Reed Army Institute of Research, and the National Center for Advancing Translational Sciences, is designed to discover new chemical agents that can be developed into prophylactic and curative medicines to protect US Military personnel exposed to malaria.



Project cascade, representing different biological and pharmacology-based assays to filter initial hits and down-select compounds. Compounds active in assay screens including *P. cynomolgi* hypnozoites *in vitro* enter iterative rounds of medical chemistry and preliminary structure-property relationship studies along with biological screens and pharmacological assessment.

Accomplishment: Identification of lead compounds to prevent and cure vivax and falciparum malaria.

Timeline and Cost

Activities CY	15	16	17	18
Aim 1 (milestone: HTS)				
Aim 2 (milestone: liver stages)				
Aims 3,4 (milestone: *in vivo* cure)				
Aim 5 (milestone: *P. cynomolgi*)				
Budget (880,000) in $	**60,000**	**260,000**	**320,000**	**240,000**

Updated: New York, October 28, 2016

Goals/Milestones

CY15 Goal – Initiate analysis of current hit compounds

X Confirm initial hits active against *P. falciparum* blood stages

CY16 Goals – Identify new hit compounds

X Screen additional 150K compounds against blood stages

X Identify liver stage-active inhibitors

CY17 Goals - Define *in vivo* active compounds

☐ Identify inhibitors active in mice, test against *P. cynomolgi in vitro*

CY18 Goal – Optimize hits, liver and blood stage efficacy

☐ Medicinal chemistry, ADMET/toxicity

☐ Test inhibitor activity in mice, *P. cynomolgi* liver stages *in vitro*

Comments/Challenges/Issues/Concerns

- None.

Budget Expenditure to Date (Columbia; not WRAIR or NCATS)

Projected Expenditure: $240,000 (began 30 September 2015

Actual Expenditure: $178,300 (as of 30 September 2016)